Cancer Causes and Control **13**: 923–928, 2002.

Toenail selenium concentration and lung cancer in male smokers (Finland)

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Received 27 February 2002; accepted in revised form 15 August 2002

Key words: lung cancer, selenium.

Abstract

Background: The objective of this investigation was to evaluate the association between toenail selenium concentration and lung cancer risk in male smokers.
Methods: We conducted a nested case–control study within the Alpha-Tocopherol Beta-Carotene Cancer Prevention Study cohort. This substudy included 250 randomly selected incident lung cancer cases and 250 controls matched on age (up to ± 5 years), intervention group assignment, and date of randomization (±15 days). Odds ratios (ORs) and 95% confidence intervals (CIs) were determined using conditional logistic regression methods. Finland began fortification of agricultural fertilizers in the fall of 1984, increasing the dietary intake, plasma, and toenail selenium concentrations for the population. The present analyses were based on the calculated residual of toenail selenium after regressing it on date of randomization. The selenium residual and the interaction of the residual with date of randomization were included in models with smoking status and body mass index as covariates.
Results: We observed a suggestion of a protective association for higher selenium status among men who entered the trial early (when the range of selenium values included very low levels). The OR for men with adjusted toenail selenium concentrations at the 75th percentile compared to those with the lowest selenium concentrations ranged between 0.20 (0.09–0.44) for men randomized earliest in the trial and 0.61 (0.27–1.41) for men randomized in the fifth year.
Conclusions: These results suggest that low selenium status may be associated with increased risk for lung cancer.

Introduction

Research has suggested that the essential trace element selenium may play a role in cancer prevention. Selenium is a key component of the glutathione peroxidase enzymes, which remove hydrogen peroxides generated *in vivo* by free radicals. Lipid hydroperoxides and their products may damage DNA and metabolically activate carcinogens [1]. There is evidence from a large randomized clinical trial to support an association between selenium and lung cancer. Clark and colleagues [2] reported that a daily 200 μg selenium supplement reduced total cancer mortality by 50% and reduced lung cancer incidence and mortality by 46% and 53%, respectively. Further, three nested case–control studies conducted in Finland using blood samples collected between 1968 and 1976 also provide support for a relationship between selenium and lung cancer [3–5].

Selenium intake varies greatly in different parts of the world, mainly because of differences in the soil selenium content. The selenium intake in Finland was estimated at 20–30 μg/day in the late 1970s and early 1980s [6], or about half the level recommended by the US National Academy of Science [7]. Finland began fortification of agricultural fertilizers with selenium in the fall of 1984 [8]. As a result average selenium intake increased in the

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next five years, stabilizing at 125 μg/day, until it was lowered in 1990 and reached approximately 80 μg/day by 1993 [9]. Plasma selenium concentrations nearly doubled during the same period, increasing from a mean of 0.89 μmol/L during the pre-fortification period to 1.52 μmol/L by 1989 [9]. Toenail selenium concentrations for two groups of Finnish middle-aged men were reported in 1984 and 1991 as 0.45 mg/kg [10] and 0.96 mg/kg [11], respectively.

Recruitment for the Alpha-Tocopherol Beta-Carotene Cancer Prevention Study (ATBC) began in 1985. We designed a nested case–control study within the ATBC Study cohort, matching cases and controls on date of entry into ATBC, to evaluate whether lower toenail selenium concentrations were associated with lung cancer risk throughout the implementation of the soil supplementation program. Based on preliminary analyses we had reason to believe that, although selenium intake overall would increase during the trial, the relative ranking of individuals would change very little.

Materials and methods

Sample population

The ATBC Study trial was conducted in Finland between 1985 and 1993 as a joint project between the National Public Health Institute in Finland and the US National Cancer Institute. The trial was a large, randomized, double-blind, placebo-controlled prevention trial to determine whether daily supplementation with α-tocopherol, β-carotene, or both would reduce the incidence of lung or other cancers. The overall design and initial results have been published [12, 13]. Briefly, 29,133 Caucasian male smokers between the ages of 50 and 69 years were recruited from southwestern Finland between 1985 and 1988 and randomly assigned to one of four groups based on a 2×2 factorial design. Men who had prior cancer, serious illnesses, or reported current use of vitamins E (>20 mg/day), A (>20,000 IU/day) or β-carotene (>6 mg/day) were ineligible. Participants received 50 mg/day α-tocopherol (as *dl*-α-tocopheryl acetate), 20 mg/day β-carotene, both α-tocopherol and β-carotene, or placebo. Active follow-up continued for 5–8 years until 30 April, 1993.

Case ascertainment and control selection

This analysis includes 250 incident lung cancer cases diagnosed prior to 30 April, 1993. Cases were identified by the Finnish Cancer Registry [14] and randomly selected within three major cell types to include approximately equal numbers of squamous (n = 90), small-cell (n = 80), and adenocarcinoma (n = 80) histological types. To enhance the ascertainment of cases a chest film was obtained at a study visit every 28 months and at each participant's exit from the study. These were matched to 250 randomly selected disease-free controls on (1) age (up to $\pm$5 years), (2) intervention group assignment, and (3) date of randomization/toenail collection ($\pm$15 days) to control for potential confounding by the selenium soil supplementation which began in 1984.

Data collection

At baseline the study participants completed a demographic and general medical history questionnaire and a food-frequency (use) questionnaire, height and weight were measured, and blood and toenail samples were collected. Toenail selenium is a reasonable indicator of selenium intake over the previous six months to one year [15].

Sample preparation and determination of selenium

For each individual, toenail samples from all toes were cleaned by treatment with 1% sodium dodecylsulfate solution for one h, ultrasonicated for one min, rinsed five times with purified water and then dried at 60 °C to constant weight. The total sample of nail clippings was cooled on dry-ice in a PTFE chamber together with a tungsten-carbide ball for about five min. The ball mill (B. Braun Biotech, Melsungen, Germany) was operated for one min at 1600 rpm. The fine powder was stored in glass vials at room temperature until analyzed. The selenium concentration of nails was determined on 10–50 mg aliquots by acid-digestion fluorimetry using 2,3-diaminonaphthalene to produce the piaselenole according to the methods of Alfthan [16]. Each batch consisted of 40 randomly ordered paired cases and controls, two different reference samples of known selenium concentration, and two randomly placed blind quality control (QC) samples of an in-house nail powder pool labeled to resemble the subject samples. The overall coefficient of variation for QC toenail selenium was 3.5% (mean 0.700 $\pm$ standard deviation (SD) 0.024 mg/kg). In these analyses the mean and SD for BCR Bovine Liver 185 was 0.467 ± 0.0089 mg/kg (certified for 0.446 ± 0.013 mg/kg) and those for EKT 96/94 liver was 0.724 ± 0.008 mg/kg (certified for 0.744 ± 0.051).

Statistical analysis

Statistical analyses were performed using Statistical Analysis Systems (SAS) software [17,18]. Dietary vari-

ables were energy-adjusted via the residual method. The characteristics of case and control subjects were compared by the Wilcoxon rank sum test for continuous variables, and chi-squared test for categorical variables. Odds ratios (ORs) and 95% confidence intervals (CIs) for the association between lung cancer and toenail selenium were determined in conditional and unconditional (controlling for the matching factors) logistic regression models. Initially toenail selenium was analyzed as a categorical variable. Because of the soil fortification program in Finland, men randomized early in the trial had the lowest selenium concentrations. The Spearman rank correlation between date of randomization and toenail selenium concentration was 0.71. Median toenail selenium concentrations for control subjects by date of randomization in the trial were 0.411 (April–December 1985), 0.450 (January–June 1986), 0.534 (July–December 1986), 0.572 (January–June 1987), 0.636 (July–December 1987), and 0.654 (January 1988 onwards) mg/kg, respectively. Therefore we conducted preliminary analyses by time of randomization, splitting the data into four time periods: (1) April 1985–June 1986, (2) July–December 1986, (3) January–December 1987, and (4) January 1988 and later. These time periods were set to include both sufficient numbers of cases and controls and also to capture changes in the population toenail selenium concentration. The toenail selenium concentrations were grouped into tertiles based on the distribution among the controls within each period. We developed 12 dummy coded indicator variables to designate both time period and tertile of selenium (*e.g.*, time period 1, tertile 1; time period 1, tertile 2; time period 1, tertile 3, *etc.*) with the earliest time period and lowest toenail selenium serving as the referent. However, the risk estimates from these models were sensitive to the dates selected as cutpoints. In follow-up analyses, presented in this article, we calculated the residual of toenail selenium after regressing it on date of randomization to adjust for the correlation between the toenail selenium and the date of randomization due to the soil fertilization program. The selenium residual and the interaction of the selenium residual with date of randomization were included in conditional logistic regression models with smoking (number of years and cigarettes per day) and body mass index (BMI, kg/m^2) as covariates. The parameter estimates and variance/covariance matrices from the model were used to calculate the point and interval estimates for the effect of toenail selenium on lung cancer risk relative to time during the study. Results are presented as ORs and 95% CIs for men at the 25th and 75th percentiles compared to men with the lowest selenium values. Effect modification by baseline serum α-tocopherol and β-carotene concentrations, age, BMI, smoking, treatment group, and alcohol intake was assessed by including the individual factor and its cross-product term with the continuous toenail selenium variable in a separate multivariate model. For interactions of interest, analyses were repeated with the variables of interest stratified and the data analyzed as unmatched, controlling for the matching factors in the models.

Results

Table 1 shows selected characteristics of lung cases and matched controls. The lung cancer cases were heavier smokers and had smoked for more years than the controls. In addition, mean BMI and total energy intake were significantly lower among cases compared to controls.

Figure 1 presents results from the multivariate conditional logistic regression model relative to time during the study. Data are presented as adjusted OR by time of randomization (year) for men at the 25th and 75th percentiles of baseline toenail selenium compared to men with the lowest toenail selenium concentrations. The OR for men with adjusted toenail selenium concentrations at the 75th percentile compared to those with the lowest selenium concentrations ranged between 0.20 (0.09–0.44) for men randomized earliest in the trial and 0.61 (0.27–1.41) for men randomized late in the trial.

There was no meaningful effect modification for the association between lung cancer and toenail selenium status by serum α-tocopherol or β-carotene, age, BMI, smoking, alcohol intake, or β-carotene trial supplement use. A marginally significant interaction between α-tocopherol treatment and toenail selenium was observed ($p = 0.07$), with a protective association apparent among the group of men who received the α-tocopherol treatment. The OR for men on α-tocopherol at the 75th percentile compared to those with the lowest selenium concentrations ranged between 0.33 (0.20–0.83) for men randomized earliest in the trial and 0.43 (0.18–1.04) for men randomized in the last year.

Discussion

Previous studies of selenium and lung cancer risk have been inconclusive. In a large, recently completed randomized trial, investigators reported that oral administration of 200 μg of selenium per day reduced risk for lung cancer risk by 46% and mortality by 53% [2]. Of the prospective studies which have looked at selenium levels

Table 1. Selected characteristics of lung cases and controls (means and standard deviations)

	Case subjects (n = 250) mean (SD)	Control subjects (n = 250) mean (SD)
Age (years)	63.0 (4.7)	63.0 (4.6)
BMI (kg/m^2)[a]	25.5 (3.8)	26.5 (4.1)
Smoking		
Cigarettes (/day)[a]	21.9 (8.4)	19.8 (9.0)
Years[a]	40.8 (7.0)	37.4 (7.7)
Dietary intake[b]		
Energy (kcal)[a]	2715 (805)	2882 (788)
Selenium (μg)	98 (30)	98 (31)
Vitamin E (mg)	15.7 (19.5)	15.1 (16.0)
Alcohol (g)	18.0 (20.5)	17.7 (21.7)
Biomarkers		
Serum β-carotene (μg/L)	207 (152)	218 (164)
Serum α-tocopherol (mg/L)	11.6 (2.6)	11.8 (2.8)
Toenail selenium (mg/kg)	0.537 (0.129)	0.550 (0.134)
Treatment group (no. subjects)		
β-Carotene	70	70
α-Tocopherol	54	54
Both	66	66
Placebo	60	60

[a] Cases significantly different from controls ($p < 0.05$).
[b] Dietary variables adjusted for energy intake via the residual method.

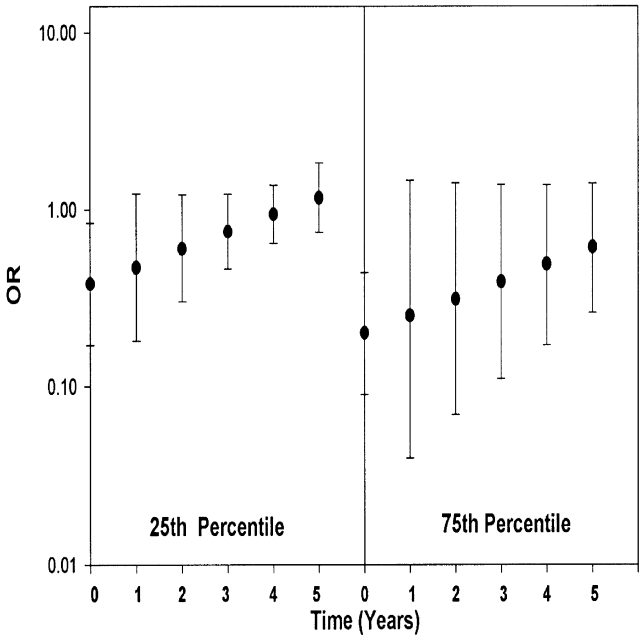

Fig. 1. Adjusted odds ratio (OR) and 95% confidence interval (CI) of lung cancer by baseline toenail selenium concentration and time of randomization. *Matched on age, intervention group, and date of randomization and adjusted for smoking and BMI.

in plasma or serum, three of 15 found significant inverse associations with lung cancer [3–5]. The other twelve [9, 19–29] did not find statistically significant differences between cases and controls. Two other prospective studies evaluated the relationship between toenail selenium status and lung cancer. One found a statistically significant inverse association [30] and another found a non-significant inverse association [31]. In all four investigations where a significant inverse association has been found, the population group had a low selenium intake overall. In the most recent of these the authors found that the effects of selenium on lung cancer risk were modified by smoking [3]. An inverse association between selenium and lung cancer was observed among smokers but not among non-smokers. These authors suggested that selenium may exert its protective effect only at low levels of selenium in relation to the oxidative stress [3]. Our results in a population of middle-aged smoking men are consistent with this hypothesis.

In the present study toenail selenium concentration tended to be inversely associated with lung cancer among those who were randomized in the earliest time period. There are several possible reasons for this result. First, this group includes the majority of those men with the lowest toenail selenium concentrations. If selenium does exert a measurable protective effect only when those with very low levels are available for comparison, then this is the group in which we would most likely

observe a protective effect. Second, toenail selenium content within the earliest time periods is likely to most closely approximate long-term intake. Toenails collected a longer time period after the implementation of the fortification are less likely to represent the true long-term intake of the individual. However, we evaluated the available dietary data to determine to what extent the fortification program might result in "misclassification" of long-term intake. The Spearman correlation between calculated selenium intakes based on a 1986 selenium database and a 1984 selenium database was 0.87. These data suggested that, for dietary selenium intake at least, the relative ranking of individuals would not change dramatically. Thirdly, it is possible that fortification lowered lung cancer risk overall; and that within the range of intakes in the later time periods the relationship between selenium and lung cancer is modified. It is unclear from the available experimental data whether the chemopreventive effects of selenium are phase-specific; however, in a recent review, Coombs and Gray [1] noted that, in animal models, anti-tumorigenic effects of selenium seem to occur during both the initiation and promotion phases of carcinogenesis. Thus it might be possible to change lung cancer risk overall in a relatively short time frame.

We observed that α-tocopherol supplementation modified the reduced risk observed for increasing toenail selenium status and lung cancer, with the effects seen among those who received α-tocopherol. Although small numbers dictate caution in interpreting this finding, synergism between selenium and vitamin E has been proposed and observed in experimental models [1].

In summary, the results of this study suggest that, at low levels of intake, there is a reduced risk of lung cancer with increased levels of toenail selenium. The association between selenium and lung cancer was weakened during later years, possibly due to the change in the overall distribution of intakes, limitations of a single toenail sample to accurately reflect long-term intake due to the fortification, or to a change in overall risk for lung cancer with the fortification. Further investigations into a possible threshold effect for selenium and the joint effects of vitamin E and selenium in the prevention of cancer are needed.

Acknowledgements

This work was supported by Public Health Service contract N01 CN 45165 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services.

References

1. Combs GF Jr, Gray WP (1998) Chemopreventative agents; selenium. *Pharmacol Ther* **79**: 179–192.
2. Clark LC, Combs GF, Turnbull BW, *et al*. (1996) Effects of selenium supplementation for cancer prevention in patients with carcinoma of the skin. *JAMA* **276**: 1957–1963.
3. Knekt P, Marniemi J, Teppo L, Heliovaara M, Aromaa A (1998) Is low selenium status a risk factor for lung cancer? *Am J Epidemiol* **148**: 975–982.
4. Knekt P, Aromaa A, Maatela J, *et al*. (1990) Serum selenium and subsequent risk of cancer among Finnish men and women. *J Natl Cancer Inst* **82**: 864–868.
5. Salonen JT, Salonen R, Lappetelainen R, Maenpaa PH, Alfthan G, Puska P (1985) Risk of cancer in relation to serum concentrations of selenium and vitamins A and E: matched case–control analysis of prospective data. *BMJ* **290**: 417–420.
6. Varo P, Koivistoinen P (1981) Annual variation in average selenium intake in Finland: cereal products and milk as sources of selenium in 1979/80. *Int J Vit Nutr Res* **51**: 79–84.
7. Food and Nutrition Board. (1998) *Recommended Dietary Allowances*, 11th edn. Washington, DC: National Academy of Sciences.
8. Varo P, Alfthan G, Huttunen JK, Aro A (1994) National selenium supplementation in Finland – effects on diet, blood and tissue levels, and health. In: Burk R, ed. *Selenium in Biology and Human Health*. New York: Springer, pp. 197–218.
9. Alfthan G, Neve J (1996) Selenium intakes and plasma selenium levels in various populations. In: Kumpulainen JT, Neve J, eds. *Natural Antioxidants and Food Quality in Atherosclerosis and Cancer Prevention*. Cambridge: Royal Society of Chemistry, pp. 161–167.
10. Ovaskainen M-L, Virtamo J, Alfthan G, *et al*. (1993) Toenail selenium as an indicator of selenium intake. *Am J Clin Nutr* **57**: 662–665.
11. Alfthan G, Kumpulainen J, Aro A, Miettinen A (1992) Selenium status of rural subjects consuming their own produce six years after onset of addition of selenate to fertilizers in Finland. *Selenium in Biology and Medicine, 5th International Symposium on Selenium Proceedings*. Nashville, TN, 20–23 July.
12. Alpha-Tocopherol Beta-Carotene Cancer Prevention Study Group (1994) The effect of vitamin E and beta carotene on the incidence of lung cancer and other cancers in male smokers. *N Engl J Med* **330**: 1029–1035.
13. Alpha-Tocopherol Beta-Carotene Cancer Prevention Study Group (1994) The Alpha-Tocopherol, Beta-Carotene Lung Cancer Prevention Study: design, methods, participant characteristics, and compliance. *Ann Epidemiol* **4**: 1–10.
14. Hakulinen T, Kenward M, Luostarinen T (1989) *Cancer in Finland in 1954–2008: incidence mortality and prevalence by region*. Cancer Society of Finland publication no. 42. Helsinki: Finnish Foundation for Cancer Research.
15. Morris JS, Stampfer MJ, Willett W (1983) Dietary selenium in humans; toenails as an indicator. *Biol Trace Elem Res* **5**: 529–537.
16. Alfthan G (1984) A micromethod for the determination of selenium in tissues and biological fluids by single-test-tube fluorimetry. *Anal Chem Acta* **165**: 187–194.
17. SAS Institute (1996) *SAS/STAT Software Changes and Enhancements through Release 6.11*. Cary, NC: SAS Institute, pp. 807–884.
18. SAS Institute (1996) *SAS/STAT User's Guide Version 6*, 4th edn. Cary, NC: SAS Institute, pp. 1–1685.
19. Peleg I, Morris S, Hames CG (1985) Is serum selenium a risk factor for cancer? *Med Oncol Tumor Pharmacother* **2**: 157–163.

20. Menkes MS, Comstock GW, Vuilleumier JP, Helsing KJ, Rider AA, Brookmeyer R (1986) Serum beta-carotene, vitamins A and E, selenium, and the risk of lung cancer. *N Engl J Med* **315**: 1250–1254.
21. Nomura A, Heilbrun LK, Morris JS, Stemmermann GN (1987) Serum selenium and the risk of cancer by specific sites: case–control analysis of prospective data. *J Natl Cancer Inst* **79**: 103–108.
22. Ratnasinghe D, Tangrea J, Forman M, *et al.* (2000). Serum tocopherols, selenium and lung cancer risk among Chinese tin miners. *Cancer Causes Control* **11**: 129–135.
23. Willett WC, Polk BF, Morris JS, *et al.* (1983) Prediagnostic serum selenium and risk of cancer. *Lancet* **2**: 130–134.
24. Kabuto M, Imai H, Yonezawa C, *et al.* (1994) Prediagnostic serum selenium and zinc levels and subsequent risk of lung and stomach cancer in Japan. *Cancer Epidemiol Biomarkers Prev* **3**: 465–469.
25. Comstock GW, Alberg AJ, Huang HY, *et al.* (1997). The risk of developing lung cancer associated with antioxidants in the blood: ascorbic acid, carotenoids, alpha-tocopherol, selenium, and total peroxyl radical absorbing capacity. *Cancer Epidemiol Biomarkers Prev* **6**: 907–916.
26. Virtamo J, Valkeila E, Alfthan G, Punsar S, Huttunen JK, Jarvinen MJ (1987) Serum selenium and risk of cancer. *Cancer* **60**: 145–148.
27. Kok FJ, van Duijn CM, Hofman A, Vermeeren R, De Bruijn AM, Valkenburg HA (1987) Micronutrients and the risk of lung cancer (Letter). *N Engl J Med* **316**: 1416.
28. Coates RJ, Weiss NS, Daling JR, Morris JS, Labbe RF (1988) Serum levels of selenium and retinol and the subsequent risk of cancer. *Am J Epidemiol* **128**: 515–523.
29. Ringstad J, Jacobsen BK, Tretli S, Thomassen Y (1988) Serum selenium concentration associated with risk of cancer. *J Clin Pathol* **41**: 454–457.
30. van den Brandt PA, Goldbohm RA, Van't Veer P, *et al.* (1993) A prospective cohort study on selenium status and the risk of lung cancer. *Cancer Res* **53**: 4860–4865.
31. Garland M, Morris JS, Stampfer MJ, *et al.* (1995) Prospective study of toenail selenium levels and cancer among women. *J Natl Cancer Inst* **87**: 497–505.